



Jabr, R., Salvage, S., Hatch, F., Orlowski, A., Lampe, P., & Fry, C. (2016). Regulation of gap junction conductance by calcineurin through Cx43 phosphorylation: implications for action potential conduction. *Pflügers Archiv: European Journal of Physiology*, 468(11), 1945-1955. <https://doi.org/10.1007/s00424-016-1885-7>

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Supplement to: Regulation of gap junction conductance by calcineurin through Cx43 phosphorylation: implications for action potential conduction.

By: Jabr RI, Hatch FS, Salvage SC, Orlowski A, Lampe PD, Fry CH

*Measurement of the intracellular  $[Ca^{2+}]_i$  in low-Na solution, with guinea-pig left ventricular trabeculae using intracellular  $Ca^{2+}$ - and  $Na^+$ -selective microelectrodes.*

$[Ca^{2+}]_i$  was recorded in isolated guinea-pig multicellular preparations, similar to those used to measure gap junction conductance and action potential conduction velocity in the present study. This was done using intracellular  $Ca^{2+}$ -selective micro-electrodes ( $Ca^{2+}$ -ISE) as these may be calibrated before and after use. Figure S1 shows a calibration curve of  $Ca^{2+}$ -ISEs at 37°C in a mock intracellular solution containing (mM): KCl, 120.0; NaCl, 8.0; HEPES, 10.0;  $MgCl_2$ , 1.0; EGTA, 0.5 mM, pH 7.1 with KOH.

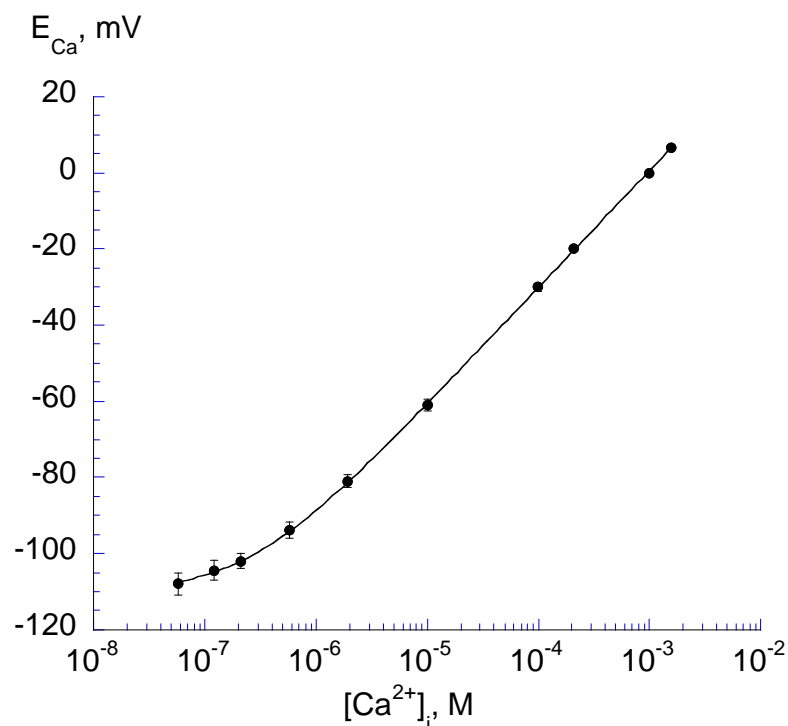


Figure S1. Calibration curve of  $Ca^{2+}$ -selective microelectrodes. Data are mean $\pm$ SD from five electrodes and normalised to  $E_{Ca}$  values at 1 mM Ca in the calibrating solution. Line is a best-fit of equation 1.

The  $[Ca^{2+}]$  of the calibrating solution was varied by adding samples of a 1 M  $CaCl_2$  stock and titrating back to pH 7.1. The  $[Ca^{2+}]$  was calculated from the method in [S1]. Figure S1 shows a best-fit line through the calibration curve as described by the modified Nikolsky equation (equation 1) where  $k_{ij}^{pot}$  is a potentiometric selectivity coefficient for  $Ca^{2+}$  (the primary ion,  $i$ ) over the major interferent ion,  $Na^+$  (labelled  $j$ ). With four electrodes  $k_{ij}^{pot}$  had a value of  $2.52 \pm 0.086 \cdot 10^{-3}$ .

$$E_{Ca} = E^0 + s \cdot \log_{10} ([Ca^{2+}] + k_{ij}^{pot} \cdot [Na^+]^2) \quad \text{eqn 1}$$

Where  $E_0$  is a constant and  $s$  is the slope constant of the electrode calibration,  $2.303RT/zF$ , where  $R$ ,  $T$ ,  $z$  and  $F$  have their usual thermodynamic meanings. In equation 1, concentration values of  $Ca^{2+}$  and  $Na^+$  have replaced activity values, but there is no loss of generality as activity coefficients will not have varied between calibration and experimental conditions as the ionic strength of the calibration and biological media were constant throughout. A calculated 'limit of detection' for  $Ca^{2+}$ ,  $[Ca^{2+}]_d$  - where the calibration slope is half the Nernstian slope, was calculated from equation 2 with a value of 0.25  $\mu M$  when the  $[Na^+] = 8$  mM.

$$[Ca^{2+}]_d = k_{ij}^{pot} \cdot [Na^+]^2. \quad \text{Eqn 2}$$

The  $Ca^{2+}$ -ISE may be used in sub-micromolar  $[Ca^{2+}]$ , but a separate determination of the intracellular  $[Na^+]$  during control and low-Na conditions was necessary to calculate the steady-state intracellular  $[Ca^{2+}]$  under these two conditions. From five experiments the intracellular  $[Na^+]$  reduced from  $7.2 \pm 1.2$  to  $2.4 \pm 0.42$  mM when going from control to low-Na conditions as used in the experiments in this paper. The characteristics of  $Na^+$ -ISEs have been described in detail previously [S2,3] and the methodology of calibration in [S1]. Therefore the steady-state concentrations of intracellular  $[Ca^{2+}]$  in

normal and low-Na solutions were estimated from  $E_{Ca}$  readings and equation S1 using the measured  $[Na^+]_i$  in the two conditions.

Figure S2 shows simultaneous outputs from a 3M-KCl microelectrode,  $E_m$ , and the  $Ca^{2+}$ -ISE,  $E_{Ca}$ , in a low-Na solution. On going from normal to low-Na solutions there was a small hyperpolarisation and an increase of  $[Ca^{2+}]_i$  that was well-sustained for the duration of the intervention. The low-Na intervention was sustained for 20-30 minutes, a similar duration to that in the experiments used to measure gap junction conductance,  $G_j$ , and action potential conduction velocity.

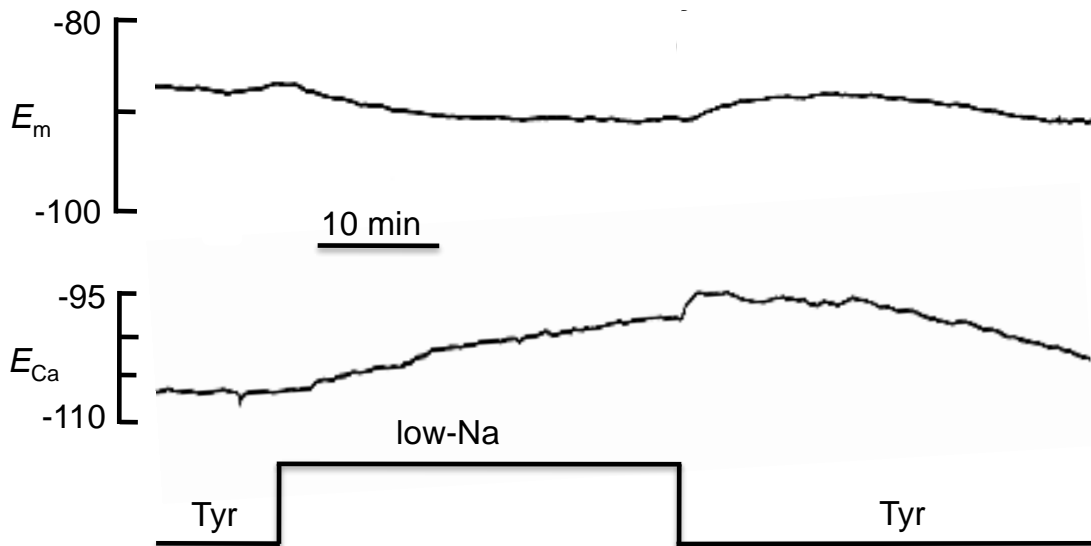


Figure S2. Measurement of membrane potential,  $E_m$ , (upper trace) and the output of a  $Ca^{2+}$ -sensitive microelectrode,  $E_{Ca}$  (lower trace, corrected for  $E_m$ ) in a guinea-pig left ventricular trabeculum. Where indicated the superfusate was changed from Tyrode's solution to low-Na (29.4 mM) solution. Experiment at 37°C. The estimated  $[Ca^{2+}]_i$  from the calibration curve, corrected for the mean change of intracellular  $[Na^+]_i$ , was from 71 to 358 nM.

It should be noted that on penetration of a cell with a  $Ca^{2+}$ -ISE the value of the electrode output (termed  $E'_{Ca}$ ) is a measure of both  $E_m$  and the  $\Delta[Ca^{2+}]_i$ , and the

separate measurement of  $E_m$  with the 3M-KCl microelectrode was subtracted from  $E'_{Ca}$  to yield values of  $E_{Ca}$ . Details of checking that the values of  $E_m$  recorded by the two microelectrodes are similar is given in S2. From four preparations the value of  $[Ca^{2+}]_i$  increased from  $85 \pm 10$  nM to  $405 \pm 105$  nM.

It is important to note that with the guinea-pig preparations the increase of  $[Ca^{2+}]_i$  was well-sustained during the low-Na intervention. This is in contrast to observations made in ferret left ventricular papillary muscles and trabeculae when only a limited transient increase of was recorded  $[Ca^{2+}]_i$  [S2]. The latter observation agrees with transient estimates of the  $[Ca^{2+}]_i$  in low-Na solution, made with micro-injection of aequorin into ferret right ventricular papillary muscles [S4,S5]. This indicates the suitability of guinea-pig preparations for the experiments carried out in the main study here and reflects variability of the handling of intracellular  $Ca^{2+}$  in different species. A further observation of the experiment in Figure S2 is the transient further increase of  $[Ca^{2+}]_i$  on return to normal-Na Tyrode's solution. This may reflect release of  $Ca^{2+}$  from mitochondria when the  $[Na^+]_i$  was to normal restored via Na/Ca exchange in this organelle [S6].

## References

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